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Reviewer: Gordon Cockell, Date June 9, 1999

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding study in the rat; OPPTS 870.4300 [§83-5]; OECD 453.

TEST MATERIAL (PURITY): CGA 293343 Technical (Thiamethoxam) 98.6%

SYNONYMS: 4H-1,3,5-Oxadiazin-4-imine,3-[(2-chloro-5-thiazolyl) methyl]tetrahydro-5-methyl-N-nitro-

CITATION: Bachmann, M. (1998) 24-Month Carcinogenicity and Chronic Toxicity Study in Rats. Novartis Crop Protection AG Toxicology, Stein, Switzerland. Test No. 942110, July 27, 1998. Unpublished. MRID 44718708.

SPONSOR: Novartis Crop Protection AG, Human Safety Assessment, Basel, Switzerland.

EXECUTIVE SUMMARY: In a combined chronic toxicity and oncogenicity study, CGA 293343, 98.6% a.i. was administered to 80 Tif: RAIf (SPF) Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 10, 30, 500 or 1500 ppm (0, 0.41, 1.29, 21.0 or 63.0 mg/kg bw/day) in males and 0, 10, 30, 1000 or 3000 ppm (0, 0.48, 1.56, 50.3 or 155 mg/kg bw/day) in females for 24 months. The animals were divided into 4 groups per dose level, with 50/sex/group used in the main chronic toxicity and oncogenicity study, 10/sex/group for hematology, clinical chemistry and urinalysis, 10/sex/group for hematology and 10/sex/group for interim sacrifice at 12 months.

Treatment with CGA 293343 had no effect on appearance and behaviour, mortality, food consumption, ophthalmology, hematology, clinical chemistry and urinalysis. Body weight gain was reduced in high-dose females during the first half of the study and water consumption was slightly increased in high-dose males. At the interim sacrifice, there were no differences observed in organ weights and gross pathology between control and treated animals. Microscopic kidney lesions were observed in males treated at 500 ppm and above. Increased incidence of lymphocytic infiltration of the renal pelvis was observed in high-dose males and a slight increase in the severity of hemosiderosis of the spleen was observed in high-dose females. At terminal sacrifice, there were no toxicologically significant changes in organ weights or gross pathology. Microscopic examination revealed increased incidence of foci of cellular alteration in the livers and chronic tubular lesions in the kidneys of high-dose females, and lymphocytic infiltration in the kidneys and chronic nephropathy in high-dose males. There was no evidence of treatment-related neoplasia in male or female rats. Dosing was considered adequate based on the observed reduction in body weight gain among high-dose females.

The LOAEL for systemic toxicity was 1500 ppm in males, equal to 63 mg/kg bw/day, based on histopathologic changes in the kidneys. **The NOAEL in males was 500 ppm, equal to 21 mg/kg bw/day**, based on the presence of regenerative kidney lesions at interim sacrifice that were not observed at terminal sacrifice.

The LOAEL for systemic toxicity was 3000 ppm in females, equal to 155 mg/kg bw/day, based on the observed reduction in body weight gain and the incidence of foci of cellular alteration in the livers and

chronic tubular lesions in the kidneys. **The NOAEL in females was 1000 ppm, equal to 50 mg/kg bw/day.**

This combined chronic toxicity/oncogenicity study in the rat is acceptable and satisfies the guideline requirement for a combined chronic toxicity and oncogenicity study (83-2); OECD 453 in the rat.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: CGA 293343 tech.

Description:	light beige powder
Lot/Batch #:	P.506006
Purity:	98.6 % a.i.
Compound Stability:	"Validity" reported as June 1999
CAS #:	153719-23-4

2. Test animals:

Species:	Rat	
Strain:	Tif: RAIf (SPF), hybrids of RII/1 x RII/2 (Sprague-Dawley derived)	
Age/weight at study initiation:	Approximately 4-5 weeks of age at delivery body weight range at week -1: males 134.7-224.7 g, females 114.2-175.0 g	
Source:	Animal Production, CIBA-GEIGY Limited, Stein, Switzerland	
Housing:	50 animals/sex/group (main study) were group housed 5/cage in macrolon type 4 cages (area 1800 sq. cm) and 30/sex/group (clinical pathology and interim sacrifice) were housed individually in macrolon type 3 cages (area 810 sq. cm)	
Diet:	Pelleted, certified standard diet (Nafag No. 8900), containing the appropriate concentrations of test material, available *ad libitum*	
Water:	Tap water, available *ad libitum*	
Environmental conditions:	**Temperature:**	22±2 °C
	Humidity:	55±10 %
	Air changes:	16-20 per hour
	Photoperiod:	12 hours dark / 12 hours light
Acclimation period:	11 days (males) and 10 days (females)	

B. STUDY DESIGN:

1. In life dates - Start: August 7, 1995 **End:** August 5-21, 1997

2. Animal Assignment/Dose Levels: Animals were assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day)	Main Study 24 months (Group I)	Hematology (Group II)	Clinical pathology (Group III)	Interim Sac. 12 months (Group IV)
Males						
Control	0	0	50	10	10	10
Low	10	0.41	50	10	10	10
Low-mid	30	1.29	50	10	10	10
High-mid	500	21.0	50	10	10	10
High	1500	63.0	50	10	10	10

Test Group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day)	Main Study 24 months (Group I)	Hematology (Group II)	Clinical pathology (Group III)	Interim Sac. 12 months (Group IV)
Females						
Control	0	0	50	10	10	10
Low	10	0.48	50	10	10	10
Low-mid	30	1.56	50	10	10	10
High-mid	1000	50.3	50	10	10	10
High	3000	155	50	10	10	10

3. **Dose Selection:** Doses were selected based on the results of the 3-Month Oral Toxicity Study in Rats (Administration in Food), CIBA project no. 942089.

4. **Diet preparation and analysis:** Diet was prepared every 4 weeks by mixing appropriate amounts of test substance with with pulverized diet (Nafag No. 8900) and was stored in stainless steel containers at room temperature until used. Analysis for homogeneity of test diets was conducted at the beginning of the study. Samples were taken from the beginning, middle and end of discharge from the pelleting machine for homogeneity analysis. Stability analysis was conducted on diets prepared for the first 4 weeks of the study after 5 weeks storage at room temperature and on diets prepared for the feeding period starting on study day 225 after 7 weeks storage at room temperature. Concentration analysis of each of the test diets was conducted 13 times over the course of the study.

Results - Homogeneity Analysis: The homogeneity of the test diets ranged from -8% to +7% of the mean concentrations.

Stability Analysis: CGA 293343 technical was found to be stable in the diets when stored at room temperature over a period of 35 days. After 35 days of storage at room temperature, concentrations ranged from 95.7% to 102.7% of nominal concentrations. Stability of the test diets was also confirmed after storage at room temperature for 7 weeks. The concentrations of diets stored for 49 days ranged from 99.2 to 108.3% of nominal concentrations.

Concentration Analysis: Actual concentrations of test diets ranged from 89.3-116.9% of nominal values. The mean concentrations for the entire study period were 99.5, 103.7, 101.3, 101.1, 100.1 and 101.1% of nominal for the 10, 30, 500 (males), 1000 (females), 1500 (males) and 3000 (females) ppm dose groups, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5.. **Statistics** - For body weight, food and water consumption, clinical pathology and organ weight data, univariate statistical analyses were performed at each time point. Non-parametric methods were applied to allow for normal and non-normal data distribution. Each treated group was compared to control using either Lepage's or Wilcoxon's two-sample test, and assessed for increasing or decreasing trend from control up to the respective dose group using Jonckheere's test for ordered alternatives. Two-sided asymptotic p-values were provided in the summary results, along with flags for significant

differences between groups and increasing or decreasing trends in the treated groups. The default level of significance to flag results from both the Lepage/Wilcoxon and Jonckheere tests was $p<0.01$.

Survival analysis was conducted using Cox's regression model to compare survival time of treated versus control animals. Analysis of the pathology data was conducted using a SAS procedure MULTTEST. Neoplastic lesions were analysed by Peto's mortality-prevalence test, non-neoplastic lesions used Cochran-Armitage's linear trend test. To account for survival differences between groups, the data were classified into the following strata: weeks 1-52, 53-78, 79-91 and 92-end. The flag for significance was set at $p<0.05$.

C. METHODS:

1. **Observations:** Animals were inspected daily for signs of toxicity and mortality.

2. **Body weight:** Animals were weighed weekly for the first three months, and monthly thereafter.

3. **Food consumption and compound intake:** Food consumption for each animal was determined weekly for the first three months and monthly thereafter. Mean daily diet consumption was calculated and reported as "food consumption ratio", with units of g food/kg body weight/day. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the food consumption and body weight gain data.

4. **Water consumption:** Water consumption was recorded monthly.

5. **Ophthalmoscopic examination**: Eyes were examined pretest and at the end of the study on all animals in the main study. Additionally, the eyes of control and high-dose animals were examined at 6, 12 and 18 months.

6. **Hematology, Clinical Chemistry and Urinalysis:** Hematology investigations were carried out on all surviving animals of experimental groups II and III (20 animals/sex/group). Urinalysis and clinical chemistry investigations were carried out on all surviving animals of experimental group III (10 animals/sex/group). Analyses were conducted at weeks 13, 27, 53, 78 and 105. For terminal investigations at week 105, the number of animals examined was supplemented by animals of the main study to yield 20/sex/group for hematology and 10/sex/group for urinalysis and clinical chemistry. Additionally, at week 105, blood smears were prepared from all surviving animals. Blood was withdrawn from the orbital sinus following an overnight fast. Urine was collected overnight, with the animals housed in individual metabolism cages. Food and water were withheld during the period of urine collection. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Red cell volume distribution width (RDW)
X	- Prothrombin time	X	Hemoglobin conc. distribution width (HDW)

* Min. req'd for carcinogenicity studies (control and high-dose only unless effects observed based on subdivision F guidelines).

b. Clinical Chemistry*

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Serum alanine amino-transferase (also SGPT)		
X	Serum aspartate amino-transferase (also SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Not required for carcinogenicity studies based on Subdivision F Guidelines.

c. Urinalysis*

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood (erythrocytes)
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

* Not required for carcinogenicity studies based on Subdivision F Guidelines.

8. **Sacrifice and Pathology:** After 12 months of treatment, the animals assigned to experimental group IV (interim sacrifice) were sacrificed and exsanguinated under ether anaesthesia. At the end of the treatment period, all surviving animals from experimental groups I, II and III were sacrificed and exsanguinated under ether anaesthesia. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The tissues from animals assigned to experimental groups I and IV were subjected to microscopic examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Peripheral nerve*
X	Esophagus*	X	Sternum with bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Axillary/mesenteric lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		**GLANDULAR**
X	Ileum*		**UROGENITAL**	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Orbital gland (Harderian)
XX	Liver*++	X	Epididymides	X	Zymbal gland
	Gall bladder*	X	Prostate	XX	Parathyroids*++
X	Pancreas*	X	Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries*+		**OTHER**
X	Trachea*	X	Uterus*	X	Bone (femur with joint)*
X	Lung*	X	Vagina	X	Skeletal muscle*
X	Muzzle			X	Skin*
				X	All gross lesions and masses*
				XX	Exsanguinated body weight

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. **Clinical signs of toxicity:** There were no differences in appearance and behaviour between treated and control animals. The author noted that in particular, there was no increase in the number of clinically palpable masses, nor in the number of animals with multiple masses.

2. **Mortality:** Mortality was not affected by treatment with CGA 293343. The survival of animals of experimental group I is presented in the table below.

TABLE 2: Survival rates of animals in main oncogenicity study

Dose	Survival to study termination	
	Males	Females
Control	32/50 (64%)	29/50 (58%)
10 ppm	37/50 (74%)	35/50 (70%)
30 ppm	24/50 (48%)	31/50 (62%)
500 ppm	32/50 (64%)	
1000 ppm		27/50 (54%)
1500 ppm	25/50 (50%)	
3000 ppm		34/50 (68%)

B. Body weight and body weight gain: Treatment with CGA 293343 tech. did not affect body weight and body weight gain in males. Decreased body weight gain was observed in high-dose females, starting in week 3 and continuing until approximately week 58. Body weight gain in high-dose females over the latter half of the study was comparable to controls.

TABLE 3: Mean body weights (BW) and body weight gains (BWG)

MALES Dose (ppm)	0	10	30	500	1500
Initial BW	190.3	191.2	190.6	190.7	191.5
BWG Wk -1-3 (% Control)	125.6	126.6 (101)	128.7 (102)	127.9 (102)	124.0 (99)
BWG Wk -1-14 (%Control)	272.9	272.0 (100)	276.2 (101)	280.7 (103)	279.0 (102)
BWG Wk 14-26 (% Control)	63.8	66.4 (104)	68.3 (107)	71.0 (111)	72.1 (113)
BWG Wk 26-54 (% Control)	93.6	100.1 (107)	98.7 (105)	106.4 (114)	115.7 (124)
BWG Wk 54-75 (% Control)	56.8	61.5 (108)	44.6 (79)	60.2 (106)	44.8 (79)
Overall BWG Wk -1-103	479.8	484.3 (101)	473.2 (99)	467.8 (97)	472.0 (98)
FEMALES Dose (ppm)	**0**	**10**	**30**	**1000**	**3000**
Initial BW	144.7	145.9	145.4	143.5	145.8
BWG Wk -1-3 (% Control)	64.9	66.3 (102)	63.9 (98)	62.8 (97)	57.5 (89)*-
BWG Wk -1-14 (%Control)	141.0	141.8 (101)	136.1 (97)	137.5 (98)	126.3 (90)*-
BWG Wk 14-26 (% Control)	30.6	27.9 (91)	31.9 (104)	31.3 (102)	26.4 (86)
BWG Wk 26-54 (% Control)	54.7	64.8 (118)	55.7 (102)	63.6 (116)	44.8 (82)
BWG Wk 54-75 (% Control)	37.3	41.7 (112)	34.0 (91)	51.2 (137)	38.4 (103)
Overall BWG Wk -1-103	284.7	291.4 (102)	281.5 (99)	299.5 (105)	248.8 (87)

Data obtained from pages 100, 101, 120 and 121 in the study report.
* Significantly different ($p < 0.01$) from the control, Lepage's test.
+/- Significant positive or negative trend ($p < 0.01$), Jonckheere's test.

C. Food consumption and compound intake:

1. Food consumption: There were no treatment-related differences in food consumption between treated and control groups at any time during the study. Time-weighted average food consumption ranged from 97-104% of control values.

2. Compound consumption (time-weighted average): Overall mean intake for males, corrected for the analytically determined concentration of the test material in the diets were 0.41, 1.24, 20.8 and 62.9 mg/kg bw/day for the 10, 30, 500 and 1500 ppm dose groups, respectively. Overall mean intake for females, corrected for the analytically determined concentration of the test material in the diets were 0.48, 1.56, 50.3, and 155 mg/kg bw/day for the 10, 30, 1000 and 3000 ppm dose groups, respectively.

D. Water consumption: There was a tendency to higher water consumption among high-dose males, with overall mean water intake from week 1-102 increased by 13% in high-dose males. There were no other differences in water consumption between control and treated groups.

E. Ophthalmoscopic examination: There were no differences between control and treated animals that could be attributed to treatment with CGA 293343 tech.

F. Blood analyses:

1. Hematology: There were no treatment-related changes observed in the hematological investigations performed at weeks 13, 27, 53, 78 and 105. A number of spurious findings were noted in males and females, but these were either not observed consistently over time, did not occur in a dose-related manner, or were slight changes with no toxicological significance. The majority of the differences that were statistically significant were well within the range of historical control values.

2. Clinical Chemistry: There were no treatment-related changes observed in the clinical chemistry investigations performed at weeks 13, 27, 53, 78 and 105. A number of spurious findings were noted in males and females, but these were either not observed consistently over time, did not occur in a dose-related manner, or were slight changes with no toxicological significance. The majority of the differences that were statistically significant were well within the range of historical control values.

3. Urinalysis: There were no treatment-related differences in the urinalysis investigations at any time during the study.

G. Sacrifice and Pathology:

1. Organ weight - **Interim sacrifice:** After 12 months of treatment, there were no differences in absolute organ weights and organ-to-body weight ratios between control and treated animals.

Terminal sacrifice: A slight decrease in exsanguinated body weight was apparent in high-dose males and females, however the difference was not statistically significant. A marginal increase in thyroid weight relative to body weight was observed in high-dose males, however the author dismissed this finding due to the absence of any histopathological correlate and because the relative weight was within the range of historical control values. In addition, the mean control value was skewed upward by one extremely high value. Comparison of median values shows the marginal increase that was observed at the high dose (see table 4). Absolute thyroid weight was increased in group 4 females, and thyroid weight relative to body weight was increased in group 3, 4 and 5 females. In the absence of corroborative clinical laboratory or microscopic findings, the study author dismissed this finding as not toxicologically relevant. The reviewer concurs with the study author.

TABLE 4: Mean absolute and relative thyroid weights at terminal sacrifice

Males - Dose (ppm)	0	10	30	500	1500
- absolute thyroid weight (mg)	111.3	73.81	63.44	69.37	61.66
- relative to body weight (%)	0.0172	0.0122	0.0102	0.0117	0.0104+
(median value)	0.0089	0.0089	0.0093	0.0099	0.0099
Females - Dose (ppm)	**0**	**10**	**30**	**1000**	**3000**
- absolute thyroid weight (mg)	45.59	49.50+	48.67	60.80*+	49.82
- relative to body weight (%)	0.0113	0.0124	0.0123+	0.0153*+	0.0136*+

Data obtained from pages 345-348 of the study report
* Significantly different from the control, $p<0.01$, Lepage's test
+ Significant positive trend, $p<0.01$, Jonckheere's test

2. Gross pathology - **Interim sacrifice:** After 12 months of treatment, there were no differences in macroscopic observations between control and treated animals.

Terminal sacrifice: A number of findings occurred more frequently in treated males and/or females. The study author concluded that the "histologic counterparts of these macroscopic findings were not indicative of an effect due to the treatment". The incidence of the specific findings are presented in table 5. The study author dismissed a slight increase in the incidence of brain deformation in high-dose males as occurring with similar frequency in control and treated animals. Microscopically, this was associated with hydrocephalus and pressure atrophy. The author stated that the increased incidence of hydrocephalus was secondary to pituitary adenoma, which was not related to treatment. The reviewer concurs with the study author.

TABLE 5: Incidence of macroscopic findings at terminal sacrifice

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
Organ/observation	**Males (n = 70)**					**Females (n = 70)**				
Liver - cyst	2	2	2	4	1	2	2	7	3	7
Lung - mottled	4	5	5	7	7	3	4	6	10	8
Pancreas - mass/nodule	4	11	6	11	6	2	1	3	0	3
Abdomen - mass	1	2	0	0	5	0	0	0	0	2
Brain - deformation	6	6	4	3	9	5	7	5	10	6

Data obtained from page 55, 374, 380, 384 and 390 of the study report.

3. Microscopic pathology:

a) **Non-neoplastic - Interim sacrifice:** Treatment-related findings were restricted to the kidneys, renal pelvises and the spleen. Cholangiofibrosis and inflammatory cell infiltration were observed more frequently in the liver of treated males, and tubular casts and tubular atrophy were observed more frequently in the kidneys of treated males, however these findings did not occur in a dose-related manner and were considered incidental by the study author. Treatment-related findings are presented in table 6, and include increased incidence of lymphocytic infiltration of the kidneys in males at 500 ppm and above and chronic tubular lesions in males at 500 ppm and above. The incidence of basophilic proliferation of renal tubules, when considered in conjunction with the chronic tubular lesions indicates an increase in total regenerative tubular lesions in male kidneys at 500 ppm and above. Lymphocytic infiltration of the renal pelvis was observed more frequently in high-dose males, and a slight increase in the severity of hemosiderosis of the spleen was observed in high-dose females.

TABLE 6: Non-neoplastic findings at interim sacrifice

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
Organ/observation	**Males (n = 10)**					**Females (n = 10)**				
Kidney - lymphocytic infiltration	1	1	0	2	3	0	2	0	0	0
- chronic tubular lesion	2	2	2	4	6	1	1	1	2	0
- basophilic proliferation	0	1	0	2	1	0	0	2	0	0
Total regenerative tubular lesions	2	3	2	6	7	1	1	3	2	0
Renal pelvis - lymphocytic infiltration	1	0	0	1	3	0	1	0	0	0
Spleen - hemosiderosis grade 1	0	0	0	0	0	0	0	0	0	0

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
-hemosiderosis grade 2	6	5	3	7	4	7	5	5	6	1
-hemosiderosis grade 3	3	3	6	2	4	2	5	5	3	8
-hemosiderosis grade 4	0	0	0	0	0	1	0	0	0	1
-Average grade	2.3	2.4	2.7	2.2	2.5	2.4	2.5	2.5	2.3	3.0

Data obtained from page 59 of the study report.

Terminal sacrifice: The author reported the following treatment related findings: increased incidence of foci of cellular alteration in the livers of high-dose females (mainly clear cell); lymphocytic infiltration in the kidneys of males (only treatment-related at 1500 ppm); and, chronic nephropathy in high-dose males. A number of other findings were reported by the author as not related to treatment with CGA 293343. The specific findings are presented in table 7. In the reviewer's opinion, it appears that the incidence of chronic tubular lesions in the kidneys of females treated at 1000 ppm and above may be related to treatment. The incidence of hydrocephalus in high-dose males was dismissed by the study author as secondary to pituitary adenoma, which was not considered to be a treatment-related finding. The reviewer concurs with the study author. The study author considered the incidence of bronchiolo-alveolar hyperplasia to be secondary to accumulation of foam cells and minimal inflammatory changes in the lung. The latter findings occurred in similar incidences in control and treated animals with no association to treatment, hence the author's conclusion is acceptable.

TABLE 7: Non-neoplastic microscopic findings at terminal sacrifice

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
Organ/observation	**Males (n = 50)**					**Females (n = 50)**				
Findings reported by the study author as treatment-related										
Liver - focus of cellular alteration	20	21	15	21	20	10**	21**	12	15	26**
Kidney - lymphocytic infiltration	10**	10	7	14	17	2	3	4	2	2
- chronic nephropathy	30**	35	32	37	42**	12	10	8	6	10
Findings reported by the study as not related to treatment										
Liver - hepatocyte hypertrophy	2	5	5	5	3	5	5	4	5	7
- biliary cyst	2	2	1	4	1	3	1	7	3	3
Kidney - chronic tubular lesion	10	10	9	6	4	14*	16	13	18	21
- cyst	2	3	1	5	2	2	1	0	1	1
- tubular hyaline change	0	1	1	0	2	2	1	0	0	0
Lung -bronchiolo-alveolar hyperplasia	0	1	1	0	2	0*	0	1	2	3
- foam cells	21	34**	33**	25	27	29	38*	28	31	26
Large intestine - dilatation	0	0	1	0	0	0*	0	0	0	2
Urinary bladder - inflammatory cell infiltration	2	0	0	0	2	0*	0	0	0	2
Thymus - chronic inflammation	0	0	0	0	0	0*	0	0	0	2
Axillary lymph node - chronic reactive hyperplasia	29	30	27	34	33	36	38	36	36	41

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
Ovaries - cystic/papillary hyperplasia						13	13	14	22*	18
Eyes - inflammation with fibrosis	0	0	0	0	0	0*	0	0	2	2
Brain - hydrocephalus	4*	3	4	2	10	4	2	5	7	5
- mineralization	0	0	0	0	0	0	0	0	0	2

Data obtained from page 62-65 of the study report.
* $p < 0.05$; ** $p < 0.01$. All analyses used the Exact trend test (more precise than the Cochran-Armitage) and the Fisher's Exact test for pair-wise comparisons. Analyses conducted by EPA statistician.

b) **Neoplastic - Interim sacrifice:** There were no treatment-related neoplastic findings at interim sacrifice. Sporadic occurrence of malignant neoplasms included malignant lymphoma in one male at 10 ppm, observed at week 46, malignant oligodendroglioma in one male at 10 ppm, observed at week 47 and mammary adenocarcinoma in one female at 1000 ppm, observed at week 28. Sporadic occurrence of benign neoplasms included fibroma of subcutaneous tissue in one control male, observed at week 45, fibroadenoma of the mammary gland in one male at 500 ppm, observed at week 53, adenoma of the pars distalis of the pituitary in one male at 1500 ppm at week 43, one control female and one female at 1000 ppm at week 53, adenoma of the prostate in one male at 1500 ppm at week 53 and hemagioma of the mesenteric lymph node in one male at 1500 ppm at week 53.

Terminal sacrifice: There were no treatment-related neoplastic findings at terminal sacrifice. The author reported the incidence of a few tumours that occurred in slightly higher incidences in treated animals, but concluded that the findings were probably incidental and not related to treatment with CGA 293343. Historical control data were provided for the liver neoplasias, renal carcinoma, subcutaneous lipoma and malignant astrocytoma. All of these findings occur spontaneously in control animals with a similar frequency to that which was observed in the treated animals in this study. A slight increase in the incidence of adenoma of the pars distalis was observed in male and female pituitary glands. This was dismissed by the study author because it is a relatively common finding in aging rats and it did not occur in a dose-related fashion. The reviewer concurs with the author's conclusions.

TABLE 8: Neoplastic findings at terminal sacrifice

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
Organ/observation	Males (n = 50)					Females (n = 50)				
Liver - hepatocellular adenoma	0	1	0	1	1	2	0	1	2	2
- hepatocellular adenocarcinoma	0	0	1	1	0	0	0	1	0	0
- hepatocellular neoplasia	0	1	1	1	1	2	0	2	2	2
Kidney - carcinoma	0	0	0	0	1	0	0	0	0	0
Skin/subcutis - lipoma	0*	1	0	1	3	0	0	0	0	0
Pituitary - adenoma of pars distalis	14	14	17	15	19	16	18	20	16	20
Brain - malignant astrocytoma	0*	0	0	1	2	0	0	1	0	0

Data obtained from page 62-65 of the study report.
* $p < 0.05$; ** $p < 0.01$. All analyses used the Exact trend test (more precise than the Cochran-Armitage) and the Fisher's Exact test for pair-wise comparisons. Analyses conducted by EPA statistician.

III. DISCUSSION

A. Investigators' conclusions: "Administration of CGA 293343 tech. at constant dietary concentrations of 0, 10, 30, 500 and 1500 ppm (mg/kg food) to male and 0, 10, 30, 1000 and 3000 ppm to female rats (Sprague-Dawley derived) for 24 months resulted in:

- No effect on survival;
- No effects on appearance and behaviour;
- Decreased mean body weight gain in females at 3000 ppm;
- No effects on food consumption and food consumption ratios;
- Slightly increased water intake in high dose males;
- No effects on clinical laboratory parameters including hematology, clinical chemistry, and urine analyses at study weeks 13, 27, 53, 78, and 105;
- No relevant effects on absolute mean organ weights and organ to body weight ratios at the interim and final sacrifice;
- No effects on gross morphology of organs and tissues at the interim and final sacrifice;
- Histological changes to kidney morphology at the interim sacrifice (chronic tubular lesion, basophilic proliferation, and lymphocytic infiltration, and lymphocytic infiltration of the renal pelvises in males at 1500 ppm) in males treated at 500 ppm and 1500 ppm; splenic hemosiderosis in females at 3000 ppm;
- Histological changes after 24 months to kidney morphology (chronic nephropathy and lymphocytic infiltration) in males treated at 1500 ppm and to liver morphology (mainly clear cell foci of cellular alteration) in females treated at 3000 ppm;
- No evidence of neoplastic changes in males and females.

Based on body weight data, the maximum tolerated dose (MTD) has been reached at 3000 ppm for females. After two years of treatment, the NOEL (no-observable-effect-level) was 500 ppm for males and 1000 ppm for females, corresponding to mean daily intakes of 21.0 and 50.3 mg/kg body weight, respectively."

B. Reviewer comments: CGA 293343 was administered to 80 rats/sex/dose at dietary concentrations of 0, 10, 30, 500 or 1500 ppm in males, equal to daily intakes of 0, 0.41, 1.29, 21.0 or 63.0 mg/kg bw/day and 0, 10, 30, 1000 or 3000 ppm in females, equal to 0, 0.48, 1.56, 50.3 or 155 mg/kg bw/day for 24 months. Ten rats/sex/group were used for interim sacrifice at 12 months, 10 rats/sex/group were used for hematology investigations at weeks 13, 27, 53, 78 and 105, and 10 rats/sex/group were used for clinical chemistry and urinalysis investigations at weeks 13, 27, 53, 78 and 105.

Treatment with CGA 293343 had no effect on appearance and behaviour, mortality, food consumption, ophthalomology, hematology, clinical chemistry and urinalysis. Body weight gain was reduced in high-dose females during the first half of the study and water consumption was slightly increased in high-dose males. At the interim sacrifice, there were no differences observed in organ weights and gross pathology between control and treated animals. Microscopically, increased incidence of lymphocytic infiltration and chronic tubular lesions of the kidneys were observed in males treated at 500 ppm and above. Increased incidence of lymphocytic infiltration of the renal pelvis was observed in high-dose males and a slight increase in the severity of hemosiderosis of the spleen was observed in high-dose females. At terminal sacrifice, there were no toxicologically significant changes in organ weights or gross pathology. Microscopic examination revealed increased incidence of foci of cellular alteration in the livers and chronic tubular lesions in the kidneys of high-dose females, and lymphocytic infiltration in the kidneys and

chronic nephropathy in high-dose males. There was no evidence of treatment-related neoplasia in male or female rats.

The LOAEL for systemic toxicity was 1500 ppm in males, equal to 63 mg/kg bw/day, based on histopathologic changes in the kidneys. **The NOAEL in males was 500 ppm, equal to 21 mg/kg bw/day**, based on the presence of regenerative kidney lesions at interim sacrifice that were not observed at terminal sacrifice.

The LOAEL for systemic toxicity was 3000 ppm in females, equal to 155 mg/kg bw/day, based on the observed reduction in body weight gain and the incidence of foci of cellular alteration in the livers and chronic tubular lesions in the kidneys. **The NOAEL in females was 1000 ppm, equal to 50 mg/kg bw/day**.

C. Study deficiencies: None.

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